33. (amended) A method of diagnosing acute pancreatitis in a patient suspected of suffering from acute pancreatitis comprising:

(a) measuring carboxypeptidase A levels in a biological fluid sample from a patient and in a blank sample containing a specific inhibitor of carboxypeptidase A activity and detecting changes in optical density resulting from hydrolysis of a carboxypeptidase A substrate by any carboxypeptidase A in the biological fluid sample and in the blank sample; and

(b) after subtracting the level of carboxypeptidase A activity determined in the blank sample from the levels determined in the biological sample, determining whether the measured levels of carboxypeptidase A in the biological fluid sample of the patient are elevated over levels in biological fluid samples from a healthy control population.

REMARKS

Claims 1-3 are pending in the instant application. Claims 1-3 have been rejected. Claims 1-3 have been amended. Reconsideration is respectfully requested in light of these amendments and the following remarks.

I. Rejection of Claims Under 35 U.S.C. 102

Claims 1-3 were rejected under 35 U.S.C. 102(b) as being anticipated by Sugiyama et al. (US Patent 4,551,272). The Examiner suggests that this patent discloses an assay for measuring enzymatic activity of carboxypeptidase A in a sample in the presence or absence of a reaction inhibitor, that carboxypeptidase A is found in the pancreas and serum, as well as disclosing a method for measuring the activity of carboxypeptidase A where activity depends on the disease wich is present. Applicant respectfully disagrees with the Examiner's conclusions regarding this reference.

Sugiyama et al. (US Patent 4,551,272) discloses the synthesis of derivatives of dipeptide and their use for determining activity of carboxypeptidase A in biological samples such as serum. Although this patent mentions that the enzyme, carboxypeptidase A, is found in the pancreas, nowhere does this patent teach or suggest how levels of the enzyme specifically correlate with types of pancreatic disease. Therefore, contrary to the Examiner's suggestion, this patent does not teach or suggest the method of claim 3 which is a method of diagnosing a specific form of pancreatic disease. The mere fact that the enzyme can be measured

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is not sufficient teaching for correlation of levels with a disease.

Also with regard to the teachings of Sugiyama and claims 1-3 of the instant invention, and contrary to the Examiner's suggestion, nowhere does this patent teach or suggest a method for either measuring carboxypeptidase A or a method for enhancing sensitivity and specificity of an assay by measuring enzyme activity in the presence of a "specific inhibitor" of the enzyme being measured. Careful review of this prior art patent failed to reveal such teaching of an inhibitor of carboxypeptidase A that is used at the same time as a substrate for the enzyme. This prior art patent teaches only that a termination solution of 6.5 mM sodium periodide, which is not a specific inhibitor of carboxypeptidase A, can be used to create a blank. The present method is based on use of a specific inhibitor of the enzyme in conjunction with a substrate. Without teaching of use of a specific carboxypeptidase A inhibitor, Sugiyama et al. fails to teach the limitations of the claims as filed. Therefore, this patent cannot anticipate the instant claims.

However, in an earnest effort to advance the prosecution of the case, Applicant has amended the claims to specify the role of the inhibitor in the methods of the instant invention. Support for

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these amendments to the claims can be found throughout the specification as filed, in particular at page 9, lines 19-35, and page 10, lines 1-13. The patent of Sugiyama fails to teach the use of a specific inhibitor of carboxypeptidase in a blank sample and thus does not anticipate the claims as amended. Accordingly, withdrawal of this rejection is respectfully requested.

Claims 1-3 were also rejected under 35 U.S.C. 102(b) as being anticipated by Sugiyama et al. (US Patent 4,432,896). The Examiner suggests that Sugiyama et al. discloses an assay for measuring enzymatic activity of carboxypeptidase A in a sample in the presence or absence of an reaction inhibitor, that they disclose that carboxypeptidase A enzyme is a protein decomposing enzyme found in the pancreas and serum, that they disclose a method wherein the activity of carboxypeptidase A is measured, and that the activity of the enzyme depends on the disease present and its extent. Applicant respectfully disagrees with the Examiner's conclusions regarding the teachings of this patent.

Sugiyama et al. (4,432,896) discloses derivatives of hippuryl-L-phenylalanine and their use as substrates for measuring activity of carboxypeptidase A in biological fluids. Careful review of this patent reveals that nowhere does this patent teach or suggest a method for either enhancing sensitivity or specificity of an enzyme

assay or a method for measuring carboxypeptidase A activity that is the same as that of the instant invention. Further, nowhere does this patent teach or suggest that measurement of levels of carboxypeptidase A will in any way correlate with the specific disease claimed in claim 3, acute pancreatitis.

In the instant invention, the methods of claims 1-3 as filed are based on use of a specific inhibitor of an enzyme, such as carboxypeptidase A, in addition to a substrate for the enzyme whose activity is being measured. Nowhere does the prior art patent of Sugiyama teach or suggest use of a specific enzyme inhibitor in any way. Without such teaching, Sugiyama et al. fails to teach the limitations of the claims as filed. Therefore, this patent cannot anticipate the instant claims. However, as discussed *supra*, Applicant has amended the claims the specify the role of the inhibitor in the methods of the instant invention. Support for these amendments to the claims can be found throughout the specification as filed, in particular at page 9, lines 19-35, and page 10, lines 1-13. The patent of Sugiyama fails to teach the use of a specific inhibitor of carboxypeptidase in a blank sample and thus does not anticipate the claims as amended. Accordingly, withdrawal of this rejection is respectfully requested.

II. Rejection of Claims Under 35 U.S.C. 103(a)

Claims 1-3 were rejected under 35 U.S.C. 103(a) as being unpatentable over Brown et al. (1987) in combination with Talley (1990). The Examiner suggests it would have been *prima facie* obvious to one of ordinary skill in the art to measure enzyme activity in a sample wherein the enzyme can be carboxypeptidase A comprising the step of measuring enzymatic activity in the presence and absence of a specific inhibitor because Brown et al. disclose the method as claimed for measuring activity and concentration of carboxypeptidase A in a serum sample to detect pancreatitis, while Talley discloses use of a compound that strongly inhibits carboxypeptidase A. Applicant respectfully disagrees with the Examiner's conclusions.

Brown et al. (1987) discloses a method for determination of carboxypeptidase A levels in serum by use of a specific substrate, N-acetyl-phenylalanyl-3-thiaphenylalanine. Using this substrate, the reference suggests that sensitivity of the carboxypeptidase detection is increased. As acknowledged by the Examiner, nowhere does this paper teach or suggest use in the assay of a specific carboxypeptidase inhibitor as a way to increase assay sensitivity or to aid in measurement of carboxypeptidase levels. In fact, the

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paper would be read to teach that the method of the authors is optimum as provided.

Talley et al. (1990) teach a method of preparing (R)-succinic acid derivatives. The patent states that the activity of carboxypeptidase A is inhibited by one of these derivatives. Nowhere does this patent teach or suggest that this inhibitor would be useful for producing methods of detection of carboxypeptidase in biological samples that have an increased sensitivity or specificity as claimed in claims 1-3 of the instant invention.

To establish a *prima facie* case of obviousness, three basic criteria must be met. MPEP 2143. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art must teach or suggest all claim limitations. The Examiner fails to provide evidence that one of skill would be motivated to combine these reference teachings to produce the method as claimed. In fact, MPEP states that the fact that references can be combined or modified is not sufficient to establish a case of *prima facie* obviousness "unless the prior art also suggests the desirability of the combination" (*In re Mills*,

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916 F.2d 680, 16 USPQ2d 1430, *Fed. Cir.* 1990). Such a suggestion is lacking in the prior art references cited by the Examiner. Accordingly, this combination of prior art cannot render the claimed invention obvious.

However, in an earnest effort to advance the prosecution, and as discussed *supra*, Applicant has amended the claims to specify the role of the inhibitor in the methods of the instant invention. Support for these amendments to the claims can be found throughout the specification as filed, in particular at page 9, lines 19-35, and page 10, lines 1-13. The combination of references cited by the Examiner fails to teach the use of a specific inhibitor of carboxypeptidase in a blank sample and thus does not make obvious the invention of the claims as amended. Accordingly, withdrawal of this rejection is respectfully requested.

III. Conclusion

Applicant believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**."

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

The claims have been amended as follows:

1. (amended) A method of enhancing sensitivity and specificity of an assay measuring enzymatic activity of carboxypeptidase A in a biological fluid sample comprising ~~measuring enzymatic activity in the sample in the presence and absence of a specific inhibitor of the enzymatic activity:~~

(a) contacting the biological sample and a blank sample with a carboxypeptidase A substrate wherein said blank sample contains a specific inhibitor of carboxypeptidase A activity; and

(b) measuring changes in optical density resulting from the hydrolysis of the carboxypeptidase A substrate by carboxypeptidase A in the biological fluid and in the blank sample, wherein the presence of the specific inhibitor of carboxypeptidase A in said blank sample enhances the sensitivity and specificity of the assay.

2. (amended) A method of measuring carboxypeptidase A levels in a biological fluid sample comprising:

(a) contacting a biological fluid sample and a blank sample with a carboxypeptidase A substrate ~~in the presence and absence of~~

~~a carboxypeptidase A specific inhibitor~~ wherein said blank sample contains a specific inhibitor of carboxypeptidase A activity; and

(b) measuring changes in optical density resulting from the hydrolysis of the carboxypeptidase A substrate by carboxypeptidase A in the biological fluid sample ~~in the presence and absence of the carboxypeptidase A specific inhibitor~~.

3. (amended) A method of diagnosing acute pancreatitis in a patient suspected of suffering from acute pancreatitis comprising:

(a) measuring carboxypeptidase A levels in a biological fluid sample from a patient and in a blank sample containing a specific inhibitor of carboxypeptidase A activity and detecting changes in optical density resulting from hydrolysis of a carboxypeptidase A substrate by any carboxypeptidase A in the biological fluid sample and in the blank sample ~~presence and absence of a carboxypeptidase A specific inhibitor~~; and

(b) after subtracting the level of carboxypeptidase A activity determined in the blank sample from the levels determined in the biological sample, determining whether the measured levels of carboxypeptidase A in the biological fluid sample of the patient are elevated over levels in biological fluid samples from a healthy control population.